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A Contribution to the Phylogeography and Anatomy of Helminthoglyptid Land Snails (Pulmonata: Helminthoglyptidae) from the Deserts of Southern California

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Abstract.—Land snails in the family *Helminthoglyptidae* are found sparingly and locally throughout southern California's deserts. They are mostly restricted to rock outcrops and talus in partially shaded canyons where they can gain access to cooler temperatures under the rocks. Several species are known only from their type localities, and were described by shell characters only. We have endeavored to locate known species, document their reproductive anatomy and embryonic shell structure, refine knowledge of their distribution, and incorporate genetic sequencing of two mitochondrial genes (COI and 16S) to investigate evolutionary relationships in these taxa. As a "first pass" molecular study, we have established basic sequence and divergence data for 27 populations of snails in five genera: *Helminthoglypta* (subgenus *Coyote*), *Eremarionta*, *Cahuillus*, *Chamaearionta* and *Sonorelix*. Fifteen of the populations were previously unknown. We confirmed that the Salton Rift/Coachella Valley is a major biogeographic barrier for land snails, as is the north/south transition between the Colorado and Mojave deserts. Described species of *Helminthoglypta* (*Coyote*) grouped together in our phylogenetic analyses and differed from each other by 8-18% in the sequence of the COI gene, concordant with differentiating shell characters. Two previously unknown populations grouped with the *Coyote* species but their COI sequences differed from the described species by 5.7-17% suggesting they may represent undescribed *Coyote* species. Populations of *Sonorelix* from the eastern Mojave were somewhat similar genetically to *Sonorella* spp. from southern Arizona but the precise nature of any relationship between these genera remains unresolved. The remaining, previously unknown populations were genetically close to described species of *Eremarionta*, but inclusion of COI sequences of two *Cahuillus* spp. rendered the genus *Eremarionta* paraphyletic, raising questions about the validity of the names applied to some described species. In particular, the subspecies *E. rowelli bakerensis* was clearly different (>11% in COI) from *E. rowelli amboiana* and *E. rowelli acus*, and deserves elevation to at least species status. The eastern Mojave *Eremarionta* from near Pahrump, Nevada may also be an undescribed species, differing in its COI sequence from its closest described relative by 6.0%. Perhaps the most surprising result from our study was the finding of a population close to the Salton Sea that was very closely related to *E. rowelli* ssp. *bakerensis* which occurs ~200 km further north.

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This highlights the complex nature of genetic variation among geographically isolated *Eremarionta* populations across the eastern Mojave and western Colorado Deserts.

Land snails in the family Helminthoglyptidae Pilsbry, 1939 are sparsely distributed throughout the two contiguous deserts of southeastern California, the Mojave Desert and the more southern and lower elevation Colorado Desert (a subdivision of the Sonoran Desert) (Figs. 1 and 2), as well as in the arid mountain ranges that define their edges. Both deserts are characterized by basin and range topography: rocky, highly eroded arid mountains with lower slopes of gravelly alluvial fans are separated by flat sandy or gravelly expanses. The basins and flats do not provide refugia for snails. For the most part, the mountain slopes do not provide sufficient shelter for snails to survive. It is most often the scattered massive rockpiles and steep, partially shaded canyons with abundant deep talus that provide snail habitat. Topography is thus a major determinant of an extremely patchy distribution of desert snails. Climate is the other determinant, specifically the long drying process of the American Southwest that stretches back at least to the Miocene (Chapin 2008, Mulch et al. 2008). As recently as the Pleistocene, these now arid lands were cooler and moister, vegetated with grassland, chaparral and botanically complex pinyon/juniper woodland interspersed with lakes and rivers (Betancourt et al. 1990, Axelrod 1977). This drying process has isolated previously more widespread populations of snails into narrow canyons, shaded cliff bases and deep talus that provide shelter from desiccation. In these refugia, desert snails spend long periods of time in dormancy between infrequent rain events. Rainfall is concentrated in the winter months as Pacific storms, with summer monsoonal rain occasionally

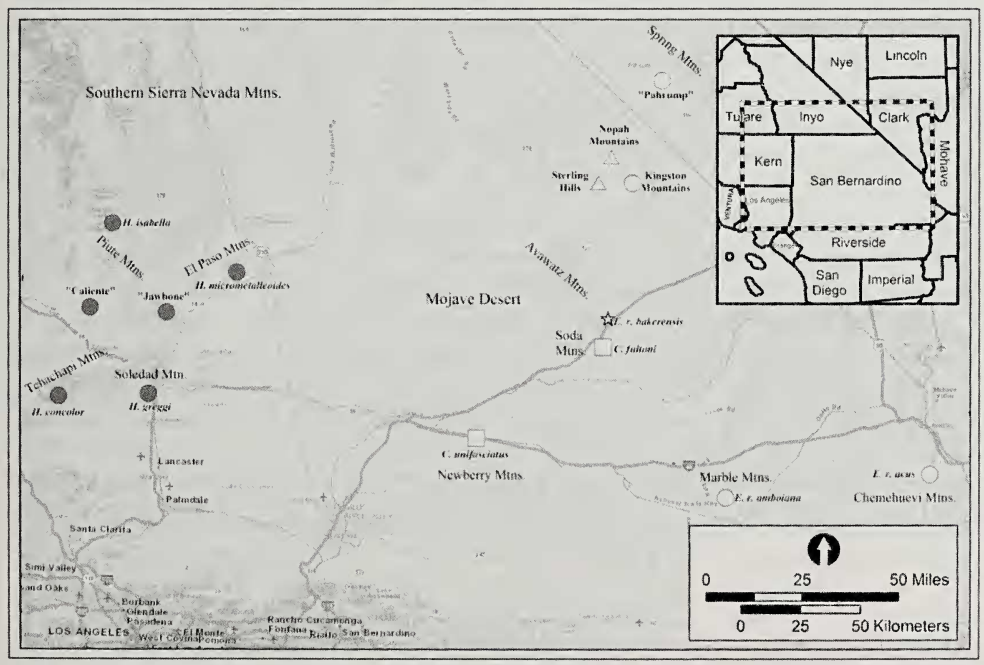

Fig. 1. Helminthoglypta (Coyote), Sonorelix, and Eremarionta/Cahuillus specimen locations map, Mojave Desert and adjacent mountains. Closed circles: Group 1. Helminthoglypta (Coyote). Triangles: Group 2. Sonorelix. Star: Group 4. *Eremarionta rowelli bakerensis* + Travertine. Open circles: Group 6. East Mojave Eremarionta. Squares: Central Mojave Cahuillus. See Appendices I and II for collection data.

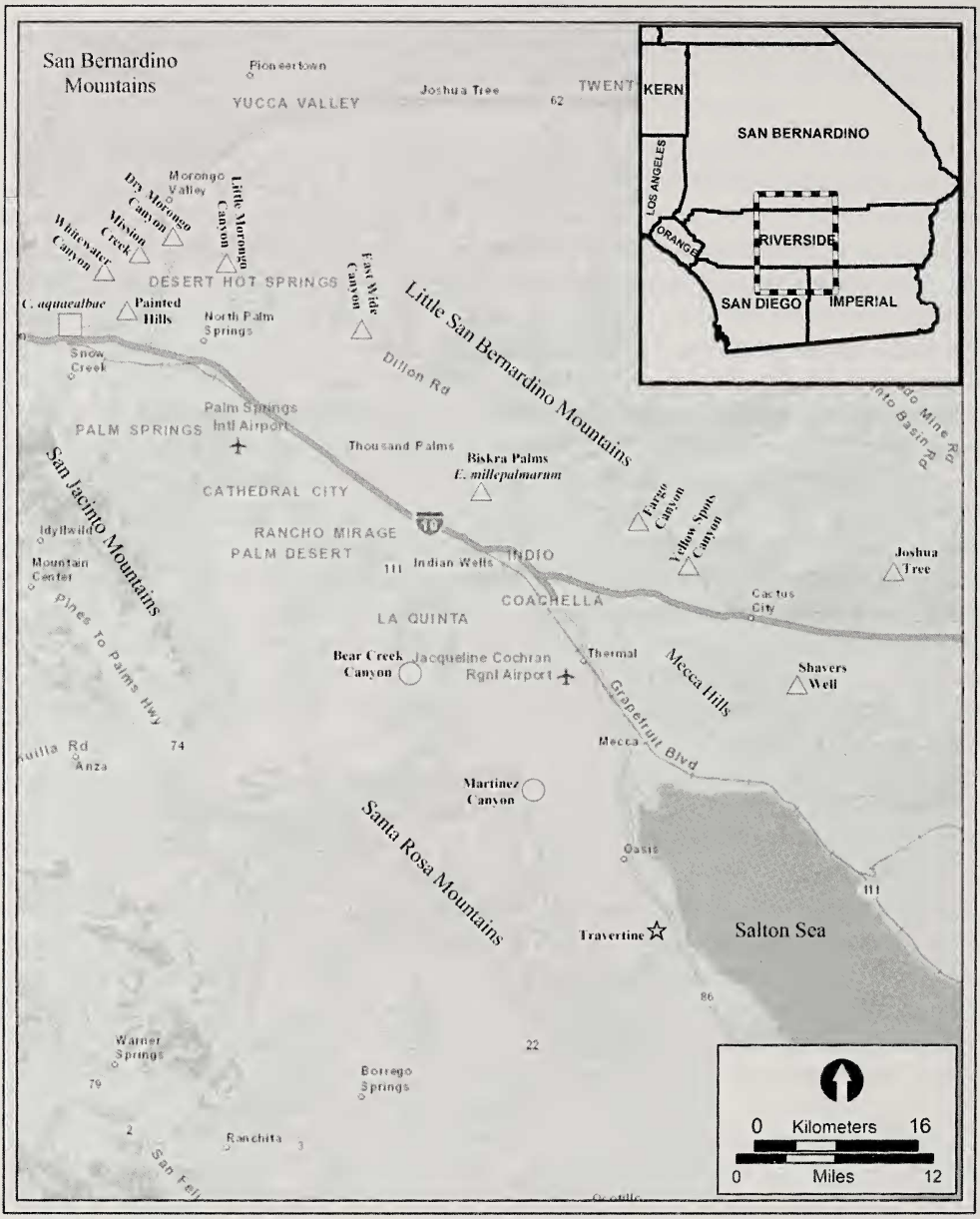

Fig. 2. Chamaearionta, Eremarionta/Cahuillus specimen locations map, Colorado Desert. Square: Group 3. *Chamaearionta aquaealbae*. Circles: Group 5. Colorado Desert Eremarionta/Cahuillus. Star: Group 4. *Eremarionta rowelli bakerensis* + Travertine. Triangles: Group 7. *E. morongoana* + *E. millepalmarum*. See Appendices I and II for collection data.

spilling over into the region from the southeast or south. We can expect varying levels of divergence between the isolated snail populations that have survived to the present. Time from separation, distance between populations, population size, selection pressure and genetic drift should all have had effects on the degree of divergence and potential speciation in the region. Stabilizing selection for ancestral characters and/or convergence have probably contributed to

the similarity in appearance of California's desert snails as diverse genetic lines were forced into the same niche, as found in seasonally arid northwestern Australia (Criscione and Köhler 2013, Köhler and Criscione 2015). Convergence on rock-dwelling habits by genetically distant *Xantusia* lizards (which often co-occur with desert snails in Arizona and California) has been noted (Leavitt et al. 2007), resulting in cryptic species.

Most of the desert snail species were described by a handful of scientists in the early 1900s, most notably S. Stillman Berry and George Willett, along with the prolific Henry Augustus Pilsbry. Their pioneering work was, of necessity, based on the snail's morphology, often of the shell only. Variability within and between isolated localities was noted and puzzled over. Later in the 20th century, the baton was picked up by Wendell Gregg, Walter Miller, Barry Roth and others who described additional species and revised the systematics of *Helminthoglypta* and other genera. We have relied heavily on *Checklist of the Land Snails and Slugs of California* (Roth and Sadeghian 2003) as well as the malacology collections at the Santa Barbara Museum of Natural History and the Natural History Museum of Los Angeles County. Recently, malacologists have been able to augment traditional taxonomic methodology with molecular markers (Gilbertson et al. 2013, Roth 2002) though most desert helminthoglyptids have yet to be sequenced.

The purpose of this study is to revisit the type localities of described species and explore new locations to update the taxonomy, anatomy and distribution of California desert snails. To explore divergence and species limits, we provide molecular data on helminthoglyptid snails in the genera *Eremarionta* Pilsbry, 1913, *Cahuillus* Roth, 1996, *Sonorelix* Berry, 1943, *Chamaearionta* Berry, 1930, and *Helminthoglypta* Ancey, 1887, using the mitochondrial genes COI and 16S. We are aware of the limitations of small sample sizes and a reliance on mtDNA only as opposed to the inclusion of nuclear DNA (e.g. Rubinoff and Holland 2005); our intent is to use mtDNA to uncover obvious inconsistencies with taxonomy, to look for cryptic taxa, and to identify groups of snails whose geography and phylogeny will require further, more detailed analysis. We present phylogenetic analyses on combined COI and 16S sequence data, but summarize sequence divergence based on COI alone since the alignment of this locus is typically unambiguous, and to allow for more consistent comparison to other taxa reported in the literature.

Our sampling focused on the western edge and the eastern portion of the Mojave Desert, and the northern and southern edges of the Coachella Valley of the Colorado Desert. See Figs. 1 and 2 for collection locations and Appendices I and II for exact collection data. Our original intent was to confine our collections to desert species, but the Jawbone Canyon *Helminthoglypta* population, despite being at the desert edge, more closely resembled foothill and montane species than the desert "*Coyotes*." Therefore, we obtained samples of the two *Helminthoglypta* subgenus *Coyote* species described from the Piute and Tehachapi Mountains that border the western edge of the Mojave Desert: *Helminthoglypta (Coyote) isabella* Berry, 1938, and *Helminthoglypta (Coyote) concolor* Roth and Hochberg, 1988. We were unable to locate *Helminthoglypta (Coyote) caruthersi* Willett, 1934 from the desert slope of the southern Sierra Nevada, which has not been found since its original description.

Materials and Methods

DNA was extracted from the excised tail tips of individual snails using a DNeasy® Blood and Tissue Kit (Qiagen, Valencia, CA) and the manufacturer's protocol. The polymerase chain reaction (PCR) was used to amplify a section of the mitochondrial cytochrome c oxidase subunit I (COI) and 16S rRNA genes. PCR was conducted in 25 μL volumes containing;

3 μL of DNA template (concentration not determined), 1X ThermoPol PCR Buffer (New England BioLabs, Ipswich, MA), an additional 1 mM $MgCl_2$, 200 μM each dATP, dCTP, dGTP, 400 μM dUTP, 4% (v/v) BSA (NEB), 1.5 U Taq polymerase (NEB), and 0.2 μM of each respective PCR primer. Primers used for COI were LCO1490 and HCO2198 (Folmer et al. 1994), and those used for 16S were 16Scs1 (5′-AAACATACCTTTTGCATAATGG-3′) and 16Sma2 (5′-CTACGGTCCTTTCGTACTA-3′) (Chiba 1999). Reactions were performed in a Mastercycler® ep gradient S thermocycler (Eppendorf North America Inc., New York, NY) with an initial denaturing step of 3 min at 95°C; followed by 38 cycles of 30 s at 94°C, 1 min at 50°C, and 1 min 30 s at 72°C; and, a final extension of 5 min at 72°C. Amplification was confirmed by agarose gel electrophoresis and PCR products were cleaned using the Wizard® PCR Preps DNA purification system (Promega, Madison, WI) and direct-sequenced in both directions at the Institute for Integrative Genome Biology, UCR.

Alignment of forward and reverse reads, and trimming of ambiguous regions from the ends of the consensus sequences, was done using SEQUENCHER 4.9 (Gene Codes Corporation, Ann Arbor, MI). The online tool, EMBOSS Transeq (http://www.ebi.ac.uk/Tools/st/emboss_transeq/) was used to translate the protein coding COI sequence into its amino acid chain, confirming the absence of indels and pseudogenes. Sequences of the COI and 16S genes from closely related and outgroup taxa were retrieved from GenBank (PopSets 451319672 and 451319700 [KC254695-722; Gilbertson et al. 2013]; and, representative sequences of several *Xerocrassa* spp. [FJ627122, FJ627139, FJ627152, JN701868, JN701871, JN701875; Sauer and Hausdorf 2012] and *Sonerella* spp. [COI only; GU344934, GU344936, GU344977, GU345023, GU345038-039; Weaver et al. 2010]). COI sequences were trimmed to match the 580bp sequences of *Sonerella* retrieved from GenBank. All sequences were concatenated, and aligned using MAFFT version 7.050 (http://mafft.cbrc.jp/alignment/software/) with default settings. The resulting matrix contained 66 terminal taxa (including outgroups), each with 1336 nucleotide positions (COI = 580 bp, 16S = 756 bp). Phylogenetic reconstruction was performed by conducting a maximum likelihood (ML) analysis in RAxML (Stamatakis 2006), using the raxmlGUI v. 1.3 (Silvestro and Michelak 2012). The GTR + Γ + I model was applied and the entire dataset was partitioned by locus and, for COI, also by third codon position. Node support was assessed from 10,000 rapid bootstrap replicates as implemented in raxmlGUI (according to Stamatakis et al. 2008). A maximum parsimony (MP) analysis was also conducted in MEGA6 (Tamura et al. 2013). A heuristic search was performed using the Subtree-pruning-Regrafting algorithm (SPR) with search level 1 in which the initial trees were obtained by the random addition of sequences (10 replicates). All positions were included in the analysis (gaps treated as missing data) and bootstrap support was assessed with 1000 replicates. Evolutionary divergence was subsequently estimated by calculating average pairwise uncorrected p-distances, based only on COI (due to its unambiguous alignment), among genetic groups identified in the ML and MP analyses (see Results), and among sample locations within those groups, again in MEGA6.

Standard shell measurements were taken as defined in (Arnold 1965). Snails in the upper range of shell diameter for the species or population were determined to be mature and therefore suitable for measuring if 1) live snails possessed a pale, swollen genital pore and/or 2) shell apertures were reflexed and/or 3) terminal growth rugae were crowded, distorted and thickened, with no intervening typical periostracal surface. Reproductive tract measurements are described and defined in association with Figs. 6 and 7. Standard calipers (SPI 2000) were used, often with a string cut to length for measuring curvatures of the organs.

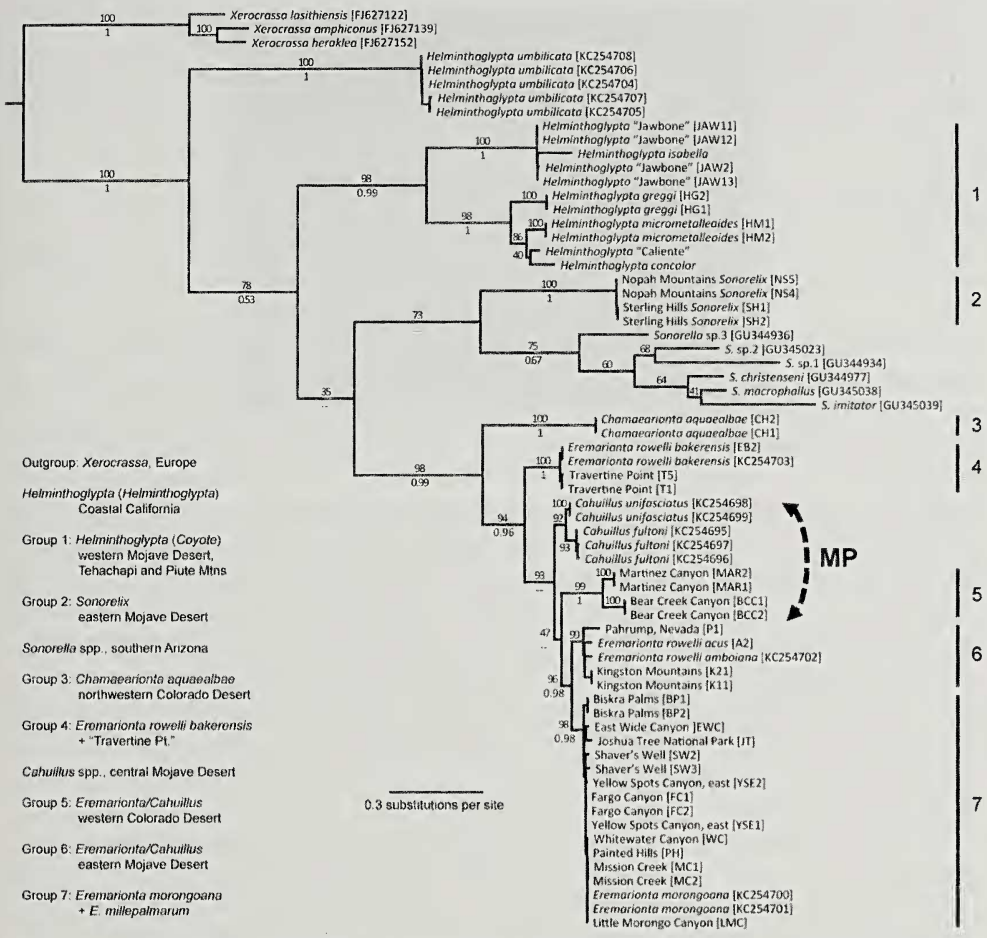

Fig. 3. Phylogenetic relationships among southern Californian land snails (Helminthoglyptidae) based on concatenated partial sequences of mtDNA COI and 16S. Maximum likelihood analysis was conducted in RAxML. ML branch support (10,000 bootstrap replicates) is shown above major branches; MP support (1,000 bootstrap replicates) below branches. Dashed arrows indicate alternative placement resulting from maximum parsimony analysis.

Results

Molecular results are presented first, followed by species accounts containing information on comparative morphology, distribution and habitat to provide a baseline characterization of each taxon within the context of the groupings shown in Fig. 3.

There was significant genetic variation across the specimens in our collections. ML analyses grouped these specimens into seven "terminal" groups (at varying evolutionary depth) each with strong support (>92%) (Fig. 3). Group 1 contained all *Helminthoglypta* (*Coyote*) spp.; group 2, all *Sonorelix* spp.; group 3, *Chamaearionta aquaealbae*; group 4, *Eremarionta rowelli* ssp. *bakerensis* + *Eremarionta* from Travertine Point; group 5, *Cahuillus/Eremarionta* from the western Colorado Desert; group 6, *Cahuillus/Eremarionta* from the eastern Mojave Desert; and, group 7, *Eremarionta morongoana* + *E. millepalmarum*. Phylogenetic relationships among those groups and the ingroup taxa *Helminthoglypta umbilicata* (Pilsbry, 1898) from central coastal California, and members of the genera *Sonorella* from Arizona, and *Cahuillus* from

Table 1. Genetic variation among *Helminthoglypta* subgenus *Coyote* snails (see Group 1, Fig. 3) based on a 580bp section of the COI gene. Mean pairwise uncorrected p-distances calculated using MEGA6.

Taxon	"Jawbone"	*isabella*	*greggi*	*micrometalleoides*	"Caliente"
isabella	0.069				
greggi	0.164	0.163			
micrometalleoides	0.169	0.179	0.116		
"Caliente"	0.168	0.164	0.093	0.057	
concolor	0.170	0.171	0.102	0.078	0.059

the central Mojave (Fig. 3), were generally well resolved with one major exception; a poorly supported branch (35%) grouping *Sonorelix* (group 2) + *Sonorella* and *Chamaearionta* + *Cahuillus/Eremarionta* (groups 3-7) as a monophyletic sister group to *H.* (*Coyote*) (group 1). Collapsing this branch results in a topology of *H. umbilicata* as a sister group to a polytomy comprised of *H.* (*Coyote*), *Sonorelix* + *Sonorella*, and *Chamaearionta* + *Cahuillus/Eremarionta*. The MP analysis resulted in a single most parsimonious tree (length 2323, r.i. 0.84, c.i. 0.48; results not shown) and recovered the same seven terminal groups each with >98% support and similar ambiguity over the exact relationships between groups 1, 2, and 3-7. However, the sister relationship between *Sonorelix* (group 2) and *Sonorella* was also unresolved creating a four-way polytomy with *H. umbilicata* as a sister group. Group 5 also switched places with the *Cahuillus* group in the MP analysis (alternative positions are indicated by arrows in Fig. 3), but the respective position of these two groups was weakly supported in both analyses (ML = 47%, MP = 72%). In light of the concordant grouping of specimens in the ML and MP analyses, levels of genetic differentiation, and characteristics of shell- and internal morphology are hereafter reported in the context of these seven groups.

Group 1 snails all belong to the subgenus *Coyote* of *Helminthoglypta*. One subgroup of *Coyote* contains *H. isabella* and the "Jawbone" snails. The corresponding subgroup contains *H. greggi* as a basal sister taxon to *H. micrometalleoides*, *H.* "Caliente" and *H. concolor*. Levels of genetic difference in the COI sequence among these six taxa (5.7 – 17.1%; Table 1) lend support to the validity of the named species, two of which were originally described on the basis of shell characteristics alone, *H. isabella* and *H. greggi*.

Group 2 consists of the genus *Sonorelix* and is genetically distant (>16%) from anything else in our sample. We encountered populations of *Sonorelix* in the Nopah Mountains and Sterling Hills in the northeastern Mojave Desert, which appear to be a single species (divergence in COI = 0.3%). ML analyses placed *Sonorelix* as a sister taxon to *Sonorella* species from southern Arizona but that relationship was lost in MP analyses. The genus *Sonorella* was not the focus of this study, but is included in our analysis because it is the most widespread, abundant, and speciose genus in neighboring Arizona (Bequaert and Miller, 1973, Miller and Naranjo-Garcia 1991). Some workers have suggested that *Sonorelix* was derived from *Sonorella* (ibid.), but based on ML and MP analyses of two mitochondrial loci we found little genetic evidence to confirm or refute this hypothesis.

Group 3 consists of the monotypic *Chamaearionta aquaealbae* (Berry, 1922). This species was strongly supported as being sister to the *Cahuillus/Eremarionta* complex (groups 4-7) in both ML and MP analyses (Fig. 3). Although genetically related to the *Eremarionta/Cahuillus* complex, *Chamaearionta aquaealbae* possesses unique reproductive and shell morphology (embryonic whorls with tightly spaced, apically ascending, elongated papillae (Fig. 4).

The remainder (and majority) of our specimens belonged to the genera *Eremarionta* and *Cahuillus*, two of the dominant snail taxa in the California deserts. Along with a group accessed

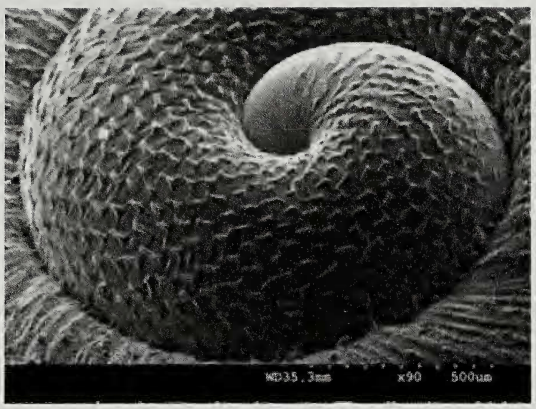

Fig. 4. *Chamaearionta aquaealbae.* SEM of embryonic whorls. SEM specimen LACM 178954.

from GenBank, four groups of *Eremarionta/Cahuillus* were identified (Fig. 3): a group comprising *E. rowelli bakerensis* and specimens from Travertine Point (group 4); a group containing specimens from Martinez and Bear Creek Canyons on the southwestern edge of the Coachella Valley, Colorado Desert (group 5); a group of specimens from the eastern Mojave comprising *Eremarionta rowelli amboiana*, *E. rowelli acus*, and previously unknown populations near to Pahrump and the Kingston Mountains (group 6); a group comprising specimens of *E. morongoana* and *E. millepalmarum* from the northern edge of the Coachella Valley (group 7); and a *Cahuillus* group comprising *C. unifasciatus* and *C. fultoni* from the central and eastern Mojave Desert (sequences from GenBank). While the *Cahuillus* group and groups 5-7 are individually well-supported, the relationships between these groups remains unresolved in the ML analysis, with only 47% support (Fig. 3). See Table 2 for COI uncorrected p-distances for groups 4-7.

Group 4 contained *Eremarionta rowelli bakerensis* (Pilsbry and Lowe, 1934), known only from the hills behind the town of Baker in the Mojave Desert. Thus, it was surprising to find a very close genetic relative 250 km away in the Colorado Desert, near Travertine Rock, at the

Table 2. Genetic variation among *Eremarionta/Cahuillus* species/populations from the Mojave and Colorado Deserts of southern California based on a 580bp section of the COI gene (see Groups 4-7, Fig. 3). Mean pairwise uncorrected p-distances calculated using MEGA6. Shaded values are of Group 6 snails. LMC (Little Morongo Canyon) is included as an example from Group 7.

Taxon (Group)	TR (4)	*bakerensis* (4)	*C. unifasciatus*	*C. fultoni*	MAR (5)	BCC (5)	LMC (7)	*acus* (6)	KI (6)	P (6)
bakerensis (4)	0.007									
Cahuillus unifasciatus	0.119	0.116								
Cahuillus fultoni	0.122	0.121	0.044							
MAR (5)	0.122	0.122	0.097	0.106						
BCC (5)	0.139	0.136	0.109	0.124	0.062					
LMC (7)	0.126	0.122	0.071	0.094	0.093	0.107				
acus (6)	0.117	0.114	0.083	0.097	0.091	0.102	0.064			
KI (6)	0.119	0.119	0.086	0.094	0.086	0.102	0.064	0.033		
P (6)	0.133	0.131	0.098	0.110	0.103	0.116	0.079	0.059	0.055	
amboiana (6)	0.126	0.124	0.086	0.094	0.084	0.109	0.062	0.029	0.026	0.060

Table 3. Genetic variation among Group 7 (*Eremarionta morongoana* and *E. millepalmarum*) populations from the northern edge of the Coachella Valley of Southern California based on a 580bp section of the COI gene. Mean pairwise uncorrected p-distances calculated using MEGA6. Populations listed west to east. WC = Whitewater Canyon; PH = Painted Hills; MC = Mission Creek; LMC = Little Morongo Canyon; EWC = East Wide Canyon; BP = Biskra Palms; FC = Fargo Canyon; YS = Yellow Spots Canyon; SW = Shaver's Well; JT = Joshua Tree National Park

Taxon (Group)	WC	PH	MC	LMC	EWC	BP	FC	YS	SW
PH	0.002								
MC	0	0							
LMC	0.002	0	0						
EWC	0.020	0.019	0.019	0.019					
BP*	0.023	0.022	0.022	0.022	0.022				
FC	0.008	0.007	0.007	0.007	0.016	0.019			
YS	0.010	0.008	0.008	0.008	0.017	0.020	0.005		
SW	0.019	0.018	0.018	0.018	0.024	0.024	0.015	0.016	
JT	0.029	0.028	0.028	0.028	0.021	0.033	0.026	0.025	0.036

* BP (Biskra Palms) = *Eremarionta millepalmarum*.

southwestern edge of the Coachella Valley (Fig. 2). COI sequences of *E. rowelli bakerensis* and the Travertine population (T) differed by only 0.7% (well within typical interspecific boundaries) and the latter is likely a disjunct population of *E. rowelli bakerensis*. By comparison, *E. rowelli bakerensis* differed from two other subspecies of *E. rowelli* included in our study by >11% (Table 2). The *Cahuillus* species from the central Mojave (accessed from GenBank) formed a monophyletic group, genetically akin to *Eremarionta*.

Group 5 consisted of two populations of *Eremarionta/Cahuillus* sampled from the western edge of the Coachella Valley at the base of the Santa Rosa Mountains: MAR = Martinez Canyon and BCC = Bear Creek Canyon (Fig. 2). These populations are expected to be closely related to *Cahuillus indioensis* (Yates, 1890), a species which is currently divided into several subspecies found along the base of the Santa Rosa and San Jacinto Mountains to the north of our collections (Roth and Sadeghian 2003). *Cahuillus indioensis* is currently under revision by LHG, DMG, and others. Genetically, the MAR population differs from that of BCC by 6.2%, yet geographically they are separated by only 16.5 km. Neither location had been sampled previously, and the specific identity of both remains unresolved.

Group 6 comprised DNA sequences of samples from three eastern Mojave Desert locations (Fig. 1): Needles (A), the Kingston Mountains (KI), and near Pahrump, Nevada (P), which grouped with *Eremarionta rowelli amboiana* from GenBank (KC254702). The Needles population is referable to *Eremarionta rowelli acus* (Pilsbry, 1939) based on geographic range, but the remaining two populations are undetermined. We currently lack detailed morphological information on the Pahrump taxon, with only one juvenile available for study. Levels of genetic difference within this group ranged from 2.6% to 6.0% (Table 2), but given the relative distances between current sample locations, defining true species boundaries will likely require a much greater sampling effort. That said, it appears that members of this group are the dominant helminthoglyptids in the eastern Mojave Desert (Fig. 1).

With the exception of *Chamaearionta aquaealbae*, all other populations from the northern and eastern edges of the Coachella Valley grouped together with sequences of *Eremarionta morongoana* from GenBank (KC254700-701) to form a final group; group 7 (Figs. 2 and 3). Genetic distances (COI) are shown in Table 3. A somewhat genetically divergent population (2.1-3.6% in COI) from the southern edge of Joshua Tree National Park (JT) also fell into this group,

which was strongly supported (>98%) in both ML and MP analyses. Another eastern population, Biskra Palms (BP), is referable to *Eremarionta millepalmarum* (Berry, 1930). Biskra Palms is the second most divergent population (1.9-3.3% in COI) but this level of genetic difference may not be sufficient to confirm that this should be treated as a different species. Genetic variation among other populations in this group peaked at 2.4% (Table 3) suggesting they are all *E. morongoana*. The Shavers Well population (SW) was originally described as *Eremarionta brunnea* (Willett 1935), but it is poorly differentiated from the other Group 7 snails. The shell morphology of snails from populations to the east of Little Morongo Canyon (LMC; Fig. 2) was not typical of those from the type locality of *E. morongoana* in Dry Morongo Canyon, and populations to the west of there. This is clearly a group that would benefit from further molecular and morphological work, perhaps leading to taxonomic revision.

Group 1, *Helminthoglypta* (*Coyote*) spp. All of the desert *Helminthoglypta* belong to the subgenus *Coyote* Reeder and Roth, 1988. This subgenus was described by Reeder and Roth, (1988) based on "a prominent bulge at the anterior end of the upper, double-tubed chamber of the penis", and a flattened, papillose shell. It is exclusively southern Californian in distribution. The type species is *Helminthoglypta (Coyote) taylori*, a narrow endemic from the desert foothills of the San Bernardino Mountains (ibid.). We sampled the type localities for *Helminthoglypta* (*Coyote*) *greggi* Willett, 1931 and *Helminthoglypta (Coyote) micrometalleoides* Miller, 1970. We were unable to obtain live specimens of *H. micrometalleoides* from Red Mountain, the only other recorded location for this species.

In group 1, two unassigned populations, "Caliente" and "Jawbone", are poorly differentiated from other group 1 snails. Shell dimensions of subgenus *Coyote* snails (Table 4) were subjected to statistical analysis in an attempt to corroborate the molecular differentiation. The "Caliente" population was excluded due to small sample size. A discriminant analysis of shell morphometrics of Group 1 snails successfully identified taxa based primarily on two functions. The first function represents a composite measure of shell size (expansion rate, diameter, height, aperture height, and aperture width), while the second is primarily based upon whorl count. Fig. 5 illustrates the nature of the differences based upon these two functions (general shell size and whorl count) for the five groups. *Helminthoglypta greggi* and *H. micrometalleoides* were readily distinguishable by the functions (Fig. 5). *Helminthoglypta isabella, H. concolor,* and the Jawbone Canyon snail have similar dimensions, and their composite functions could correctly place only a portion of their shells in the correct taxon (Table 5); 75% and 70% respectively of the Jawbone Canyon and *H. isabella* snails were correctly identified. As seen in Fig. 5 the Jawbone Canyon snail was slightly smaller than the *H. concolor* snails but larger than the *H. isabella* snails. Though larger in size, *H. concolor* did not separate cleanly in the discriminant analysis with only 50% placed correctly. This analysis should benefit from increasing the sample size.

Helminthoglypta greggi (Fig. 6): All museum records of *H. greggi* are from Soledad Mountain in Kern County. We located *H. greggi* on only two other hills near to the type locality: Standard Hill, 3 km northeast of Soledad Mtn., and the other 6 km to the west at Middle Buttes. Both hills have been extensively mined, with very little undisturbed habitat remaining. The single live snail found at Middle Buttes genetically groups closely with the Soledad Mountain snails (D. Eernisse, unpublished data). The only evidence from Standard Hill is a single shell, morphologically very similar to Soledad specimens. We repeatedly searched other nearby hills, particularly those of comparable height and area to Soledad Mountain such as Tropico and Rosamond Hills without finding any snails. *H. greggi* therefore appears to be restricted mainly to Soledad Mountain, with small populations on the two other hills mentioned above. Soledad Mountain has been and still is being extensively mined, with serious reductions in undisturbed

Table 4. Shell Measurements of *Helminthoglypta* (*Coyote*) taxa. Upper value: mean, lower value: standard deviation. Valid N listed under species name. Measurements in millimeters.

Taxon	Whorl count WC	Greater diameter GD	Height HT	Aperture height AHT	Aperture width AW	Umbilicus diameter UD	Expansion rate GD/WC
"Jawbone" (12)	**5.06** (.138)	**20.02** (1.101)	**11.02** (.818)	**10.31** (.597)	**10.37** (.935)	**3.20** (.335)	**3.96** (.184)
isabelia (10)	**5.29** (.137)	**20.51** (1.813)	**11.76** (.711)	**10.95** (1.074)	**9.87** (1.008)	**3.54** (.493)	**3.87** (.276)
greggi (11)	**5.05** (.144)	**14.15** (.545)	**7.45** (.759)	**6.24** (.361)	**6.23** (.450)	**2.73** (.294)	**2.81** (.075)
micrometalleoides (5)	**4.6** (.144)	**11.94** (.568)	**6.30** (.752)	**5.46** (.670)	**5.34** (.272)	**2.73** (.179)	**2.60** (.173)
concolor (6)	**5.23** (.137)	**21.43** (1.081)	**11.60** (.759)	**10.45** (1.34)	**10.62** (.776)	**3.40** (.400)	**4.11** (.173)

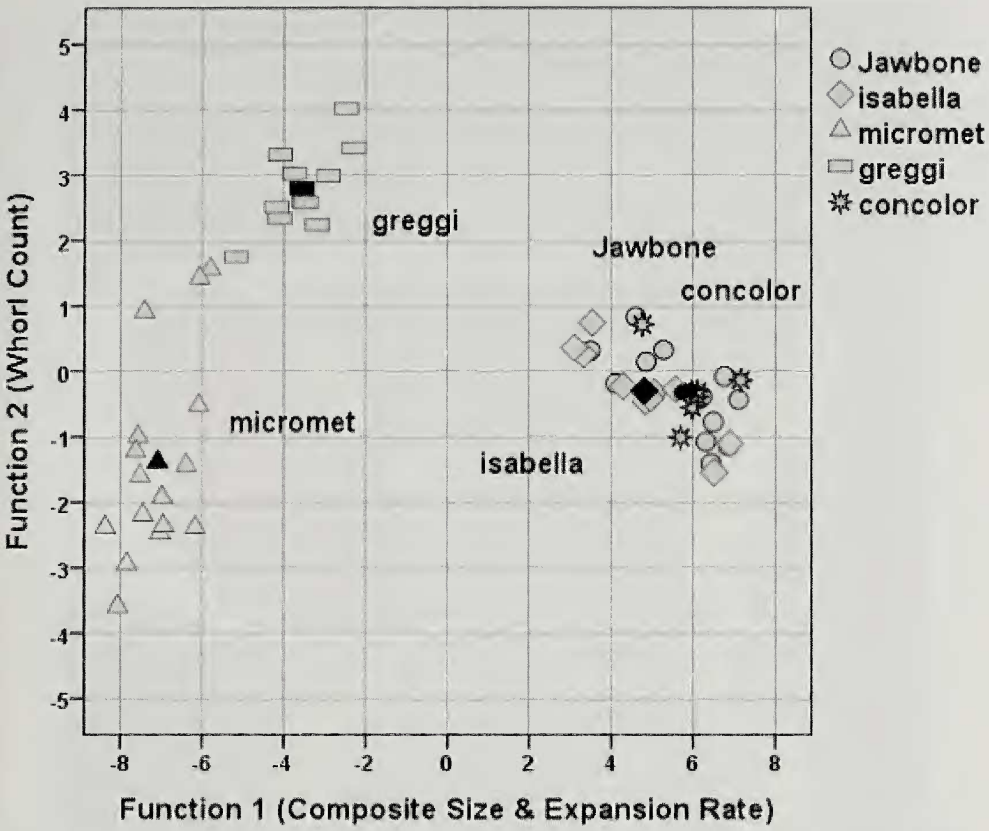

Fig. 5. Canonical Discriminant Function comparison of 5 populations of *Helminthoglypta* (*Coyote*) (Group 1). Filled symbols are the group centroids. micromet = *H. micrometalleoides.*

habitat. *H. greggi* is found in igneous rock outcrops and talus on slopes of low sparse desert scrub.

The species most similar to *H. greggi* in appearance, habitat, and type of location (small isolated ranges near the western edge of the Mojave Desert) is *H. micrometalleoides*. All our collections were from the type locality as described by Miller (1970) in Iron Canyon, in the northern El Paso Mountains. We did not acquire live samples from the only other known population of this species at nearby Red Mountain. Despite the morphological and ecological

Table 5. Predicted group membership of *Helminthoglypta* (*Coyote*) taxa by discriminant functions (Number of specimens assigned to each taxon).

Taxon	"Jawbone"	isabella	micromet*	greggi	Concolor
"Jawbone"	9	2	0	0	1
isabella	1	7	0	0	2
micromet*	0	0	5	11	0
greggi	0	0	0	0	0
concolor	2	1	0	0	3

* micromet = micrometalleoides.

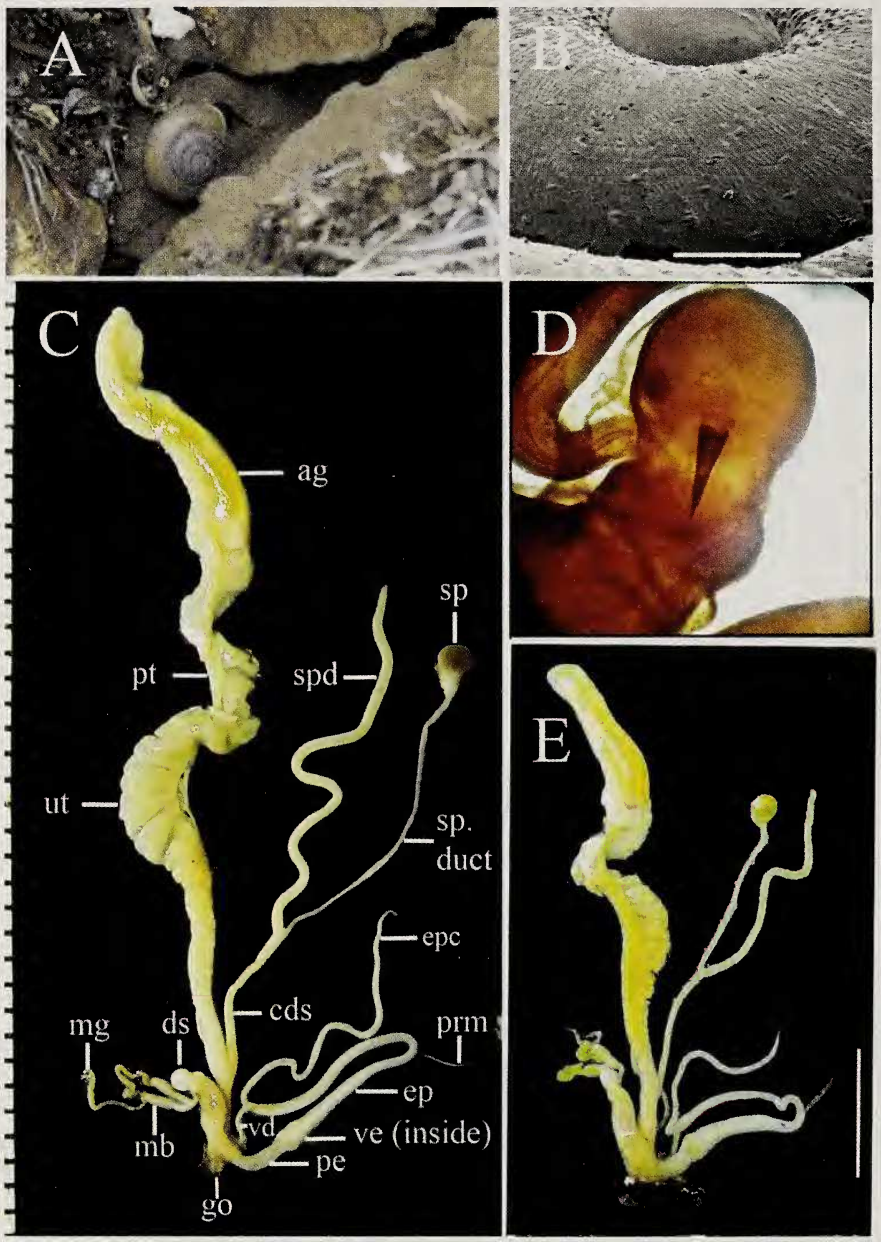

Fig. 6. A. *Helminthoglypta* (*Coyote*) *greggi,* photograph of the snail *in situ.* B. SEM of embryonic whorls of *H. greggi* (in part). Scale = 300um. C. Freshly dissected reproductive tract of *H. greggi.* Scale in mm. Measurements: Table 4. D. Stained *H. greggi* dart sac with dart. Magnification = 40X. E. Reproductive tract of *H. micrometalleoides* (at same scale as *H. greggi*). Scale bar = 5 mm. Anatomical and SEM specimens: LACM 178952 (*H. greggi*) and LACM 178953 (*H. micrometalleoides*). Abbreviations: ag, albumen gland; ds, dart sac; ep, epiphallus; go, genital orifice; mb, mucus bulb; mg, mucus gland; prm, penial retractor muscle; pt, prostate gland; ut, uterus; vd, vas deferens; ve, verge; others as in Table 6.

Table 6. Comparative reproductive morphology measurements: *Helminthoglypta* (*Coyote*) *greggi* and *Helminthoglypta* (*Coyote*) *micrometalleoides* (fresh, illustrated specimens) and *Helminthoglypta* (*Coyote*) *concolor* (slide-mounted specimen, Roth and Hochberg 1988). Measurements are to the closest 0.5 mm. Abbreviations: CDS, common duct of the spermatheca (copulatory canal); SPDU, spermathecal duct (bursa duct); SP, spermatheca (bursa copulatrix); TOT, total length of CDS + SPDU + SP; SPD, spermathecal diverticulum (bursa tract diverticulum); VA, vagina; FO, free oviduct; EPC, epiphallic cecum; SWEP, single-walled section of epiphallus (proximal region); DWEP, double-walled section of epiphallus including verge (distal region); PE, penis. Micro = micrometalleoides.

Species	CDS	SPDU	SP	TOT	SPD	VA	FO	EPC	SWEP	DWEP	PE
greggi	6.0	12.5	1.5	20.0	17.0	2.5	2.0	13.0	10.0	4.0	3.0
micro-	5.5	6.0	1.0	12.5	10.0	2.0	2.0	7.0	8.0	3.0	2.0
concolor	13.0	16.0	2.0	31.0	33.5	3.5	6.0	38.5	17.0	6.5	4.0

similarities between these two species, they are not each other's closest relatives and genetic divergence between the two species was 11.6% (Table 1).

The organs of the reproductive tract of *H. greggi* are noticeably longer overall than the organs of the smaller *H. micrometalloides* (Table 6, Fig. 6C and E). This is especially noted for the lengths of the spermathecal duct and diverticulum, as well as the epiphallic cecum. Secondly, its epiphallus is more elongated and cylindrical (i.e. not bulging as noticeably in the distal region). The mucus gland bulbs of *H. greggi* are elongated as well. Otherwise, the reproductive tracts of these two species show similar characteristics and are typical of subgenus *Coyote*.

The SEM of the embryonic whorls of *Helminthoglypta greggi* shows ornamentation with rounded elongated papillae arranged in apically ascending, well-spaced, rather ill-defined, spiral rows separated by cross-rows of numerous short rugae. (Fig. 6-B). Both the papillae and rugae are much smoother (abraded-looking) than typical for helminthoglyptids. However, the shell was fresh and immature (10.2 mm diameter) and should not have been subject to much, if any, obvious abrasion. Shell coloration in adults is tan to light brown, and body color is black fading to beige on the foot.

Helminthoglypta concolor was previously known only from the type locality, where it was found under fallen bark and logs of White Fir (*Abies concolor* (Gordon & Glend.) Lindley) (Roth and Hochberg 1988). We have expanded the known range to two additional canyons in the Tehachapi Mountains, Cottonwood and El Paso canyons, the latter being 12 km southwest of the type location in Tejon Canyon. These snails are found on north-facing conifer or mixed oak/conifer woodland slopes high on the coastal side of the Tehachapi Mountains. Elevations are higher than for any of the other *Coyote* taxa in this paper, between 1,623 and 1,803 m (5,325 – 5,912 ft). Swaths of suitable habitat are separated from each other by large tracts of chaparral and oak woodland savannah. These large, dark brown snails have rugose shells with more frequent and prominent papillae compared to *Helminthoglypta greggi* and *H. micrometalleoides*. The mantle as seen through the shell is irregularly mottled with dark spots and patches, unlike the two desert species which appear uniformly light brown with an indistinct shoulder band.

Helminthoglypta isabella is known only from the type locality, which was rather vaguely described by the collector (Berry 1938). The species still persists at the type vicinity south of Isabella Reservoir, and appears to be most abundant around the town of South Lake at a series of limestone outcrops in a predominantly granitic region. Elevations of three localities range from 939 to 1016 m (3,081-3,333 ft). It was described as being found underneath dead clumps of *Hesperoyucca whipplei* (Torr.) Baker, but it is also found under rocks and in rock crevices.

The habitat of *H. isabella* is dry rocky slopes vegetated with open chaparral with scattered oaks and pines. Its reproductive morphology is unknown.

The previously unknown "Jawbone" snail grouped closely with *H. isabella* but still differed by 6.9% in COI, and it is unclear whether or not it represents an undescribed species (Fig. 3). Shell analysis was similarly suggestive but inconclusive (Table 5, Fig. 5). Its shell is similar in appearance to that of *H. isabella* but paler in color. The "Jawbone" snail is found in narrow side canyons and talus in desert scrub in the lower portion of Jawbone Canyon, at elevations ranging from 881-922 m (2,890-3,025 ft). Indicator plants for suitable habitat are Bladdersage (*Scutellaria mexicana* (Torr.) A. J. Paton and Mormon Tea (*Ephedra nevadensis* S. Watson when suitable rock features are also present. It is a narrow endemic, with a total range of about 32 km^2, about 44 km southeast of *H. isabella* (Fig. 1).

The specific status of population "Caliente" is also unresolved. Genetically, "Caliente" is closely related to *H. concolor* and *H. micrometalleoides* (Fig. 3, Table 1), but characterizing the true relationship between these three taxa will likely require additional samples. To date, it has been found only in the steepest and shadiest portion of Caliente Canyon on the west side of the Piute Mountains, in mixed oak/Gray Pine (*Pinus sabiniana* Dougl.)/Buckeye (*Aesculus californica* (Spach) Nutt.) woodland at approximately 700 m (2,300 ft) in elevation. Caliente Canyon runs through the southwestern slopes of the Piute Mountains, about 32 km. north of the type locality for *H. concolor* in the Tehachapi Mountains (Fig. 1). The internal anatomy of the Caliente snail is unknown, but shell size and form is similar to *H. concolor*, though somewhat lighter in color with denser, more regular shell papillation. A small sample size precluded discriminant analysis of the shells.

Sonorelix (Fig. 7): This genus was described by Berry (1943) based on its lack of a dart sac and accompanying mucus glands, and an embryonic shell with anastomosing ridges. The superficially similar *Eremarionta* and *Cahuillus* usually possess dart sacs and mucus glands, and have embryonic shells ornamented with well-spaced, spirally arranged and elongated papillae. SEM imagery of the embryonic whorls of *Sonorelix baileyi* shows the reticulate (anastomosing) pattern of ridges characteristic of the genus and described as "sub-retiform" (ibid) (Fig. 7C). Based on morphology and geographical range, we consider the species sampled to be *Sonorelix baileyi* (Bartsch, 1904), the type locality of which lies on private land in Inyo County, about 8 km from our closest collection. Unfortunately, we did not have access to the type locality. The Sterling Hills site extends the known range of *S. baileyi* 6 km southward to a new station in San Bernardino County.

The reproductive tract of *Sonorelix* was described by Berry (1943) based on four taxa: (*S. borregoensis* Berry, 1929; *S. b. ora* Willett, 1929; *S. rixfordi*, Pilsbry, 1919; and *S. avawatzica* Berry, 1930). A major characteristic of these species is their lack of a dart sac and accompanying mucus glands. They exhibit a very long vagina with a unique muscular node, a long spermathecal duct with a robust diverticulum preceded by a very short common duct, a short epiphallus with a well-developed cecum, and a penis that is abruptly set off and enlarged from the epiphallus. All of these conditions are clearly shown in *S. baileyi.* However, Berry also mentioned and illustrated an excessively large "spermatotheca" (= spermatheca) and the penis containing a short conical verge. By comparison, *S. baileyi* has a moderate-sized spermatheca and an oblong, cylindrical verge (Fig. 7A and D).

Chamaearionta aquaealbae (Fig. 4): Our collections of this taxon are from the vicinity of Whitewater Canyon, for which this species is named. This canyon is located in the transition zone between the coastal slope and the northwestern edge of the Coachella Valley/Colorado Desert. The specimen sequenced was taken 6 km to the southwest of the type locality at the mouth of Cottonwood Canyon. We have extended its range by one canyon to the northeast of Whitewater,

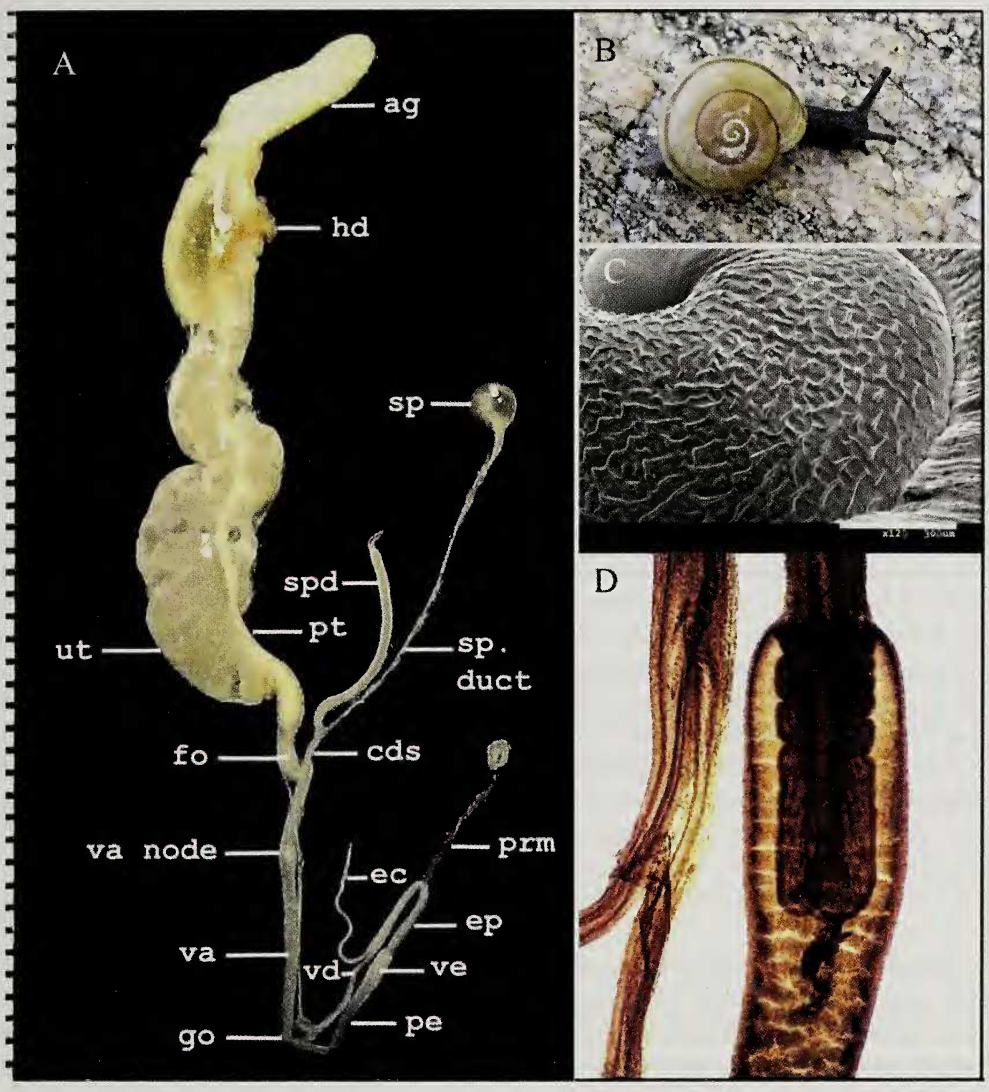

Fig. 7. *Sonorelix baileyi*. A. Freshly dissected entire reproductive tract (ex. ovotestis). Scale in mm. B. Live snail, shell diameter = 13 mm. C. SEM of embryonic whorls (in part), scale = 300um. D. Male anatomy (in part) showing verge (verge = 1.2 mm). Material leaving verge may be part of a spermatophore. Anatomical and SEM specimens, LACM 178951. Abbreviations: fo, free oviduct; ec, epiphallic cecum; others as in Table 6 or Fig. 6.

to that of Mission Creek about 4 km away. A shell from the Mt. San Jacinto foothills behind Cabezon is the only locality from the south side of San Gorgonio Pass. Whitewater Canyon is the furthest east and most desert-like station for the widespread coastal species *Helminthoglypta tudiculata* (A. Binney, 1843) as determined by museum records, and the furthest west station for *Eremarionta morongoana* (Berry, 1929) (this study). *C. aquaealbae* has been found under shrubs in leaf litter, piles of buried branches in gullies and under rocks in shaded locations.

Eremarionta/Cahuillus: The type species for *Eremarionta* appears to be *E. desertorum* (Pilsbry and Ferriss, 1908) from southwestern Arizona, though its taxonomic history is somewhat murky. The genus *Cahuillus* was recently erected from *Eremarionta* to account for a difference

in the shape and comparative length of the double-walled portion of the epiphallis (Roth 1996). The two *Cahuillus* species accessed from GenBank, *C. unifasciatus and C. fultoni,* have the characteristic anatomy of that genus (Gilbertson et al. 2013). Our sample includes mtDNA sequences from several immature snails that could not be accurately determined on morphological grounds as *Cahuillus* or *Eremarionta*, and even in adults, the distinguishing characters are often difficult to see in dissected, slide-mounted reproductive tracts. Therefore, we refer generally to Groups 4-7 snails as *Eremarionta/Cahuillus.* The named species of *Eremarionta* in our study have not been proven anatomically as belonging to one genus or the other, but to avoid premature taxonomic redesignation, are referred to as *Eremarionta*, their given name, until such time as their true generic identity is determined. The inclusion of *Cahuillus* sequences in our genetic analysis thus appears to render the genus *Eremarionta* paraphyletic, but until all of our "*Eremarionta*" are proven to possess reproductive tracts typical of the genus (and until *E. desertorum* is sequenced), this paraphyly must remain hypothetical.

Geographically, *Eremarionta/Cahuillus* are found throughout the eastern Mojave and the Colorado Deserts, with most described species and subspecies in California. New stations for Group 6 snails are the Kingston Mountains and foothills of the Spring Mountains in Nevada (Fig. 1). Additional populations will likely be found in suitable habitat in other eastern Mojave Desert mountains. *Eremarionta morongoana* (Group 7) has proven to be more widespread than previously thought, occupying a series of canyons across a range of at least 80 km (Fig. 2). All *Eremarionta/Cahuillus* occur in rockslides with deep talus surrounded by desert scrub. Some also occur at palm oases where they find shelter and moist soil under beds of fallen palm fronds, especially where tumbled rocks are also present, such as BCC = Bear Creek Canyon in Group 5 and *Eremarionta millepalmarum* in Group 7). Shell morphology and color vary subtly between the various *Eremarionta/Cahuillus*, with the basic form of a smooth, flattened, tan shell with a thin brown shoulder band and a blackish body (see Discussion for comments on convergence and conservation of shell characters).

Discussion

The use of molecular markers in systematics is now standard (Avise 2004) and has led to the discovery of previously unknown biological diversity, including cryptic species (Pfenninger and Schwenk 2007, Jockusch et al. 1998, Hanken 1999). DNA barcoding for evidence of speciation became increasingly popular (Hebert et al. 2003), in part due to the need to quickly document biodiversity in the face of environmental degradation (Vietes et al. 2009, Fouquet et al. 2007). Hebert et al. (2003) proposed a 3% divergence in COI as a suggested "universal" species lower limit. However, 3% divergence is unlikely to be universal across all taxa (Rubinoff et al. 2006).

Terrestrial pulmonate snails have proven to be particularly troublesome, and deep intraspecific divergence in the COI of these species may not be uncommon. Davison (2002) summarized the surprisingly large range of mtDNA and allozyme variation in land snails from many regions and discussed possible causes. In Davison's study, intraspecific divergence levels typically ranged from 3% to 15%. Some, but not all of the snails discussed by Davison with divergence levels greater than 10% are widespread species with very large populations, such as *Cepaea nemoralis* (Thomaz et al. 1996) and *Helix aspersa* (Guiller et al. 2001). These taxa might be expected to show high genetic variation stemming from their large population size along with local and ancient isolation of demes that subsequently diverged, as hypothesized by Thomaz et al. (1996). Of course, if one accepts the biological species concept, these diverse populations may actually represent cryptic species. Without reciprocal crossing data, it is hard to discount this alternative hypothesis.

One reason for variation in the divergence level associated with species limits is an underlying inconsistency in mtDNA substitution rates between taxa (Avise et al. 1992, Kessler and Avise 1985, Nabholz et al. 2008). Hayashi and Chiba (2000) found that divergence between clades of *Euhadra peliomphala* (Bradybaenidae) ranged as high as 9.5%. When correlated with known geological events, this translated to a higher than expected mutation rate of 10% per million years, that could only be partially explained by its complex history of colonization and isolation over a changing geographical landscape. Their conclusion was that *E. peliomphala* mtDNA mutates considerably faster than expected. Weaver et al. (2006) adopted the rapid mutation rate of 6% per million years based on the works of Yamazaki et al. (1997) and Wethington and Guralnick (2004).

Not all studies of land snails have found evidence of high mtDNA mutation rates or mtDNA divergence. For instance, Hugall et al. (2002) found similar substitution rates between vertebrates and the tropical land snail *Gnarosophia bellendenkerensis,* and Ketmaier et al. (2010) found an average intraspecific divergence level of 3.6% + −0.2% in the land snail *Solatopupa guidoni*, a level comparable to that of vertebrates. Köhler and Johnson (2012) reported on mtDNA divergence (16S, COI) in Australian land snails in the genus *Amplirhagada*. They found that about 6% divergence was both the upper limit for clear morphological species as well as the lowest interspecific distance. They suggested that in their snails, the divergence range of 5–7% indicated "young" or incompletely differentiated species, which needed to be correlated with consistent morphological differences for evidence of full speciation. Davison et al. (2009) summarized much of the published COI data for land snails. They found no consistent "barcoding gap" or threshold between intra- and interspecific taxa. Their synthesis, (using Kimura-2 parameter distances) found a mean intraspecific distance of 2.5–2.6% and a mean interspecific distance of 10.0–11.8%, but there were many instances of high intraspecific divergence as well as low interspecific divergence. We used these percentages as broad indicators of genetic distances that might denote species level differences between helminthoglyptids, but much like Köhler and Johnson (2012) and Davison et al. (ibid.) recommended, we adhere to a combined approach using DNA along with morphology to inform taxonomic decisions, as exemplified by Kelly et al (2007) for chitons.

Species limits in Helminthoglypta. – Helminthoglypta is the only genus from the snails we sampled that has previously been sequenced. Roth, Lindberg and Cordero initiated the first in-depth work on *Helminthoglypta* (subgenus *Helminthoglypta*) that synthesized morphological and molecular data. Their studies of snails in northern California and southern Oregon (contained in unpublished reports: Roth 2002,[1] Lindberg and Cordero 2002[2]) uncovered cryptic species that are clearly differentiated by molecular markers (mtDNA COI, 16S) and have validated other species previously delineated only by shell and internal anatomy characters. Roth's 2002 report shows interspecific differences ranging from 12.7% to 20.0%. While intraspecific difference was less than 2% in 5 of 6 described or proposed species, the provisional Western Trinity clade contained two specimens that differed at 6.2% and 10.7% respectively from three other samples that differed less than 0.7% from each other. Lindberg and Cordero's 2002 report analyzed essentially the same data set, and found interspecific distances of 11%–23%, and

[1] Roth, B. 2002. Unpublished memorandum. Taxonomy and Classification of *Helminthoglypta*: BLM Purchase Order HAB020406. Corvallis Forestry Sciences Laboratory.

[2] Lindberg, D., and A. Cordero. 2002. Unpublished report. Molecular phylogeny of some land snails of the clades *Monadenia* and *Helminthoglypta* in Southern Oregon and Northern California. Report to USDI BLM, Roseburg, Oregon.

typical intraspecific distances of 1.2% to 2%. Roth and Lindberg have recently begun additional analysis of their data, and Roth (pers. comm.) has indicated that as part of their new research, the variable Western Trinity clade will be analyzed anew with additional specimens. To summarize the data available at this time, most but not all analyzed *Helminthoglypta* species have an intraspecific range of 2% or less, and a lower interspecific threshold of 11–12%. "Troublesome" taxa fell in the 6.2–10.7% range.

Most of the following discussion is limited to the terminal groups from our phylogenetic analyses, which deal with subgeneric classification levels. It would be premature to use this study to comment extensively on the higher level relationships between helminthoglyptid genera, because of weak support through the center of our analyses (Fig. 3). For example, the higher level relationships of *Sonorelix* and *Sonorella* have been studied and discussed in depth due to their similarity in reproductive structure, but there is no consensus on whether they are both simplified due to homoplasy or are closely related in lineage (Miller and Naranjo-Garcia 1991, Roth 1996). In our study, their status as sister taxa received some support in ML analyses (73%), but this relationship was lost in MP analyses. A better understanding of their phylogentic position relative to each other, and to the other helminthoglyptid genera, will require a robust genetic investigation beyond the scope of our current study.

In our analyses, the *Helminthoglypta* subgenus *Coyote* is not the sister group to *Helminthoglypta sensu stricto*, as represented by *H. umbilicata*, but rather to the group containing the remainder of the included helminthoglyptid genera. This could be an artifact of limited sampling of the *Helminthoglypta s. s.*, and/or our choice of a somewhat distant outgroup taxon, *Xerocrassa*. We felt that *Xerocrassa* was the closest taxon for which comparable sequence data was readily available (in GenBank), but acknowledge that a different outgroup may have resulted in a monophyletic *Helminthoglypta*. That said, discarding the *Xerocrassa* sequences and conducting unrooted analyses also did not result in support for a monophyletic *Helminthoglypta* (data not shown). Within our *Coyote* group (Group 1), most of the geographically isolated taxa have good bootstrap support, which corroborates the current taxonomy, recognizing *H. isabella*, *H. greggi*, *H. micrometalleoides* and *H. concolor* as valid species. By accepting *H. concolor* and *H. micrometalleoides* as distinct species based on their distinct differences in ecology and shell and internal morphology (see species accounts), we set the minimum COI interspecific difference for this subgenus at 7.8% (Table 1).

The recognition of the Jawbone Canyon population as a distinct species may be warranted, but it is relatively closely related to *Helminthoglypta isabella* (6.9% different in COI) and quite similar in appearance. Unlike *H. isabella,* the Jawbone Canyon snail is a true desert dweller, but lives at the extreme edge of the Mojave Desert only 3 or 4 km from slightly higher but still fairly arid slopes similar to those occupied by *H. isabella,* about 48 km further north. Despite the partial statistical differentiation of shell morphology between *H. isabella* and the Jawbone Canyon population (Fig. 5), the general unreliability of shell morphology in the taxonomy of pulmonates casts a shadow over its utility in this case. See (Goodfriend 1984) for a summary of shell variation, and (Köhler and Criscione 2014) for examples of widespread convergence and parallelism in shell form in western Australia camaenids). For now, the specific identity of the Jawbone snail remains undetermined, at least until the internal anatomy of *H. isabella* is described, and more intervening areas in the Piute Mountains are thoroughly searched.

Helminthoglypta "Caliente" is still mostly unknown. Although genetically and morphologically similar to *H. concolor*, additional study will be required to determine its specific status. It is not unexpected to find populations such as those in Caliente and Jawbone canyons that do not fit neatly into a category of species, and could represent "young" or incipient species.

Helminthoglypta micrometalleoides presents two interesting questions. First, the genetic clustering of *H. micrometalleoides* with *Helminthoglypta concolor* is unexpected, in that the geographically closer Jawbone *Helminthoglypta* is "skipped over" (Fig. 1), suggesting a complex geological history to the common ancestors of these snails. Secondly, even though *H. micrometalleoides* is consistently the smallest of the *Coyote* snails in its natural habitat, a shell series at SBMNH of *H. micrometalleoides* from Red Mountain shows variation in shell size between small wild individuals and individuals lab-reared by Walter Miller that grew as large as *H. greggi* (but nowhere near the size of *H. concolor).* Red Mountain and the El Paso Mountains are drier and hotter than those occupied by the larger foothill and montane *Coyote* species. Such an extreme environment could be expected to have selected for smaller snails that could either delve deeper into smaller crevices, mature sooner at a smaller size, or both. The desert *Coyote* species including *H. micrometalleoides*, *H. greggi* and several central Mojave species, are notably smaller than the montane and foothill *Coyote* species. Selection for small size could be counterbalanced by retaining a degree of phenotypic plasticity to respond to more favorable environmental conditions when they occur. See (Anderson et al. 2007) for an example of environmentally induced variation in shell size.

Group 7 snails, (*Eremarionta morongoana* and *E. millepalmarum*) despite being found in a number of adjacent canyons are found only in particular microrefugia, and gene flow between canyons is extremely unlikely under current climatic conditions. Given the variation in shell form and size exhibited by these snails from different canyons, it appears that *E. morongoana* is undergoing active differentiation. The furthest west populations of *Eremarionta morongoana* are quite similar to each other in form as well as in genetic uniformity: Whitewater, Painted Hills, Mission Creek, Dry Morongo (type locality), and Little Morongo all show typical *morongoana* form (and mtDNA uniformity), while populations further east vary from location to location. We are tentatively assigning all populations of Group 7 to *Eremarionta morongoana,* including *E. millepalmarum.* While this population is somewhat more isolated geographically from the other members of Group 7, it does not differ in mtDNA sequences to an extent justifying specific status when compared to divergence levels within Group 6 and between Groups 6 and 7. If *E. millepalmarum* were to be retained as a full species, other forms such as that from Shaver's Well (SW) would warrant specific status as well, due to similar levels of genetic differentiation and distinct shell characteristics. This may indeed be appropriate, but a taxonomic decision concerning *E. millepalmarum* and other distinct forms of *E. morongoana* must wait until more morphological and nuclear molecular data is gathered.

The Little San Bernardino Mountains and Joshua Tree National Park form a portion of the north-south transition zone between the Mojave and Colorado Deserts. The Mojave/Colorado desert ecotone has been documented as a zone demarcating lineage breaks in small mammals, lizards, snakes and a toad (summarized in Wood et al. 2013), tarantulas (Graham et al. 2015) as well as the more obvious differences in vegetation and climate (Axelrod 1977). This transition zone is also evident for land snails, separating *E. morongoana* from *Cahuillus unifasciatus* to the northwest and *Sonorelix rixfordi* to the immediate north. The areas to the east of Joshua Tree National Park (Eagle Mountains, Coxcomb Mountains and further east) need to be thoroughly sampled for *Eremarionta/Cahuillus* to determine the degree of isolation and divergence between *E. morongoana* and snails in the East Mojave (Group 6), as well as other species in eastern Riverside County currently under study (Eernisse, Gilbertson, and Goodward, unpublished data). Within Group 7, the most divergent sample is the easternmost snail, from the southern edge of Joshua Tree National Park (maximum 3.8% difference in COI). Interestingly, of the Group 7 populations, this Joshua Tree snail was also the most similar to those in Group 6 (6.0% difference). Once the mountains further east are sampled, it could be that the apparent

intra-interspecific gap of 3.8% to 6.0% will disappear, replaced by clinal variation. A Mantel Test was performed on Group 7, and the p-value was significant at the 0.05 level ($p = 0.0461$, 10,000 permutations), suggesting isolation by distance is a significant factor. Concurrent to this, lineage sorting and/or limited gene flow seems to be leading to distinctive populations, such as the snails described as *E. millepalmarum*.

Eremarionta rowelli is clearly a polyphyletic taxon. Species that are (or were) under the name *rowelli* appear in groups 4, 6 and the central Mojave *Cahuillus*. In the latter group, *Eremarionta rowelli unifasciatus* was recently transferred to *Cahuillus* and elevated to species status on the basis of reproductive tract characters and genetic divergence (Gilbertson et al. 2013). As an example of the discovery of cryptic species through genetic sequencing, *E. rowelli bakerensis* plus TR (Travertine) snails (Group 4) diverge basally with strong bootstrap support from the remainder of *Cahuillus/ Eremarionta* samples. Further study (in progress, LHG) of this taxon is predicted to show that it should be removed from *rowelli* and elevated to specific status. *E. r. bakerensis*, known only from one location in the Mojave Desert clusters closely with Travertine snails from the Santa Rosa foothills, 250 km distant. This distance spans the Salton Rift, a portion of the extension zone between the Pacific and North American Plates developed in the late Miocene-Pliocene (McQuarrie and Wernicke 2005, Stock and Hodges 1989), the transition between the Colorado and Mojave Deserts, and the late Pleistocene Lake Mohave, present from 14-9 ka (Enzel et al. 2003).

Despite these isolating barriers and formidable distance, our samples of *Eremarionta rowelli bakerensis* and the Travertine Rock population are genetically very similar with regards to mtDNA markers (0.7% difference). If these two populations are remnants of a previously widespread species that has been sundered and reduced in range, we would still expect a higher degree of divergence similar to those exhibited by all the other sampled taxa. Without direct evidence, we are reluctant to invoke recent relocation by humans or other vertebrates as a possible explanation for the genetic similarity, but it is mentioned as a possibility since there is precedent for this process in Europe (Jesse et al. 2011, Grindon and Davison 2013).

Conclusions

Desert and foothill land snails in the family Helminthoglyptidae have proven to be more widespread than the literature and museum collections would indicate. Nearly all taxa are allopatric and occur in isolated microhabitat patches. All of the desert snails are found in the same basic climate regime and habitat type (talus and rock piles), and do not exhibit differentiating food preferences, at least in captivity. Mitochondrial DNA sequencing revealed hidden diversity with a wide range of genetic distances. Over comparable geographic distances, *Helminthoglypta* (*Coyote*) taxa tend to have greater genetic distances between them than *Eremarionta* and *Cahuillus*. The wide range of genetic distances with no consistent "barcoding gap" made it difficult to make taxonomic assignments. In the subgenus *Coyote*, named species based on morphology were corroborated with mtDNA analysis, but two newly discovered populations were ambiguous in both genetics and morphology. In the genera *Eremarionta* and *Cahuillus*, some taxa appear different in morphology yet are close in genetic distance, while others are genetically distinct but morphologically similar. It is unclear what species concept might be the most appropriate model for these helminthoglyptids. To test the biological species concept, reciprocal crossing trails would of necessity be conducted under artificial conditions, potentially bypassing behavioral reproductive barriers, and would be technically challenging since these snails often take four or more years to mature in captivity under natural seasonal activity patterns (pers. obs. DG). We recommend further study of *H. micrometalleoides*, including additional collections of

the population at Red Mountain and rearing experiments to replicate those of Walter Miller to further explore plasticity in shell size.

Observations on courtship and mating would be useful in determining how selective these snails are during their extremely limited activity periods. Sequences of nuclear genes or microsatellites could help determine if introgression has taken place or if lineages have been consistently isolated, and help clarify potential instances of paraphyly uncovered in this study, as well as the relationships between genera that still remain unclear. Additional morphological work is needed as well to fully elucidate paraphyly, particularly between *Eremarionta* and *Cahuillus*.

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Appendix I. Material Examined and Collection Data

Specimens are listed by genetic group (see Fig. 3), then alphabetically by taxon within the group. The numbers listed in parentheses following the taxon names are the numbers of specimens examined or collected per lot. Within each taxon, museum specimens utilized for measurements are listed first, as indicated by museum accession numbers.

LACM = Los Angeles County Museum (Natural History Museum of Los Angeles County) SBMNH = Santa Barbara Museum of Natural History.

Newly collected specimens are listed next. These are in the collections of Goodward and Gilbertson and will be deposited at LACM and SBMNH. Collector is D. Goodward unless indicated otherwise. Specimens with names enclosed in quotes are of uncertain taxonomy. Museum vouchers of newly collected material are listed separately in Appendix II.

Group 1: *Helminthoglypta concolor* (1): holotype, SBMNH 34947, "Tejon Canyon, 10.3 road mi E of cemetery; in White Fir deadfalls", 03/07/1987. (2): paratypes, SBMNH 34950, 34948, same collection data as preceding. (2): under White Fir bark, El Paso Canyon, Tehachapi Mountains, Kern Co., CA. 34° 56' 52"N 118° 36' 26"W, 03/09/2015. (2): under White Fir bark, Cottonwood Canyon, Tehachapi Mountains, Kern Co., CA. 34° 57' 51"N 118° 36' 07"W, 03/09/2015. (2): GenBank accessions pending, "under White Fir bark and rock, vic. type locality, Tejon Canyon, Tehachapi Mountains, Kern Co., CA. 35° 0' 59"N 118° 30' 09"W, 03/09/2015. collector Mike White.

Helminthoglypta greggi (2): topotypes, SBMNH 128018, "Soledad Peak" 11/26/1944.

(2): topotypes, LACM G-2534, "3.5 miles south of Mohave, Kern Co. CA." 11/26/1944.

(1): SBMNH 11907, "hill 3 $\frac{1}{2}$ mi. S of Mojave; in rock slide" 11/29/1931. (2): SBMNH 142729, "N. slope of Soledad Mt.; under rocks" 11/26/1944. (2): SBMNH 11909,11908, "North slope Soledad Mt.; under rocks" 11/26/1944. (3): SBMNH 72926, "Soledad Mt, 3-4 mi S of Mojave, in rockslides along North slope" 11/16/1957. (6): LACM G-2535, "Under rocks, north slope of Soledad Mountain, Kern County California" 11/26/1944. (10): LACM G-7596, "Under rocks, north slope of Soledad Mountain, Kern County, Calif" 11/16/1957. (1): GenBank KY986341, north slope of Soldedad Mtn., Kern Co., CA. 34° 58' 50.6"N 118° 10' 41.5"W, 03/09/2012. (1): GenBank KY986340, museum voucher, see Appendix II.

Helminthoglypta isabella (3): SBMNH 72586, "along Highway 178, 3.8 miles NE of crossing with Kernville road at Isabella" 3/3/1957. (2): LACM G-2662, "under dead yucca, 2 mi. east of Isabella, Kern Co., CA, 9 June 1945." (1): LACM G-7497, "under dead yucca, south of highway 178, 3.8 miles east of New Isabella, Kern Co., CA, 3 November 1957." (4): 1.5 mi. south of Hwy.178, South Lake, Kern Co., CA. 35° 37' 14"N 118° 22' 06"W, 08/28/2015. (1): GenBank accession pending, Squirrel Valley, Mountain Mesa, Kern Co., CA. 35° 36' 34"N 118° 24' 12"W, 12/09/14.

Helminthoglypta "Jawbone" (3): GenBank KY986336, KY986337, KY986338, northern base of White Mountain, Jawbone Canyon, Kern Co., CA. 35° 17' 46"N 118° 08' 47"W, 4/12/12. (1): GenBank KY986339, Jawbone Canyon, Kern Co., CA. 35° 18' 49"N 118° 05' 04"W, 04/27/12. (1): Jawbone Canyon, Kern Co., CA. 35° 18' 49"N 118° 05' 04"W, 06/24/13. (2): Jawbone Canyon, Kern Co., CA. 35° 18' 59"N 118° 05' 02"W, 12/04/14. (1): Jawbone Canyon, Kern Co., CA. 35° 18' 07"N 118° 05' 30"W, 03/04/14. (2): Jawbone Canyon, Kern Co., CA. 35° 18" 49"N 118° 05' 06"W, 12/04/14. (6): Jawbone Canyon, Kern Co., CA. 35° 17" 21"N 118° 06' 46"W, 03/15/15.

Helminthoglypta "Caliente" (1): GenBank accession pending, Caliente Creek Rd. 6.3 mi. east of Bodfish turnoff, Kern Co., CA. 35° 18' 25"N 118° 29' 38"W, 12/3/14.

Helminthoglypta micrometalleoides (5): SBMNH 6885, "Red Mountain; in rockslides on N. side of a northern spur, near town off US 395." 12/16/1977. (11): type locality, Iron Canyon, El Paso Mountains, Kern Co., CA. 35° 26' 36"N 117° 47' 32"W, 10/24/2015.

(2): Specimen #1: GenBank KY986342, type locality, Iron Canyon, El Paso Mountains, Kern Co., CA. 35° 26' 36"N 117° 47' 32"W, 04/13/2013. Specimen #2: museum voucher, same collection data as preceding, listed in Appendix II.

Group 2: *Sonorelix baileyi* (2): Specimen #1: GenBank KY986344, museum voucher, listed in Appendix II. In talus, south side of Old Spanish Trail, Emigrant Pass, Nopah Mountains, Inyo Co., California, 35° 53' 10"N 116° 04' 13"W. 19 December 2012. Specimen #2: GenBank KY986345, same location and date as above. (2): GenBank KY986346, KY986347, Narrow canyon just west of abandoned limestone mine, S of West Talc Road, Sterling Hills, San Bernardino Co., CA.

35° 47' 20"N 116° 07' 45"W, 02/09/12.

Group 3: *Chamaearionta aquaealbae* (2): Specimen #1: GenBank KY986349, Under *Chilopsis linearis* trees, access road adjacent to Cottonwood Wash, 1.3 km NNE of Haughen-Lehmann Way Exit, I-10, Riverside Co., 33° 56' 08"N 116° 41' 12"W. 9 April 2013. Specimen #2: GenBank KY986348, museum voucher listed in Appendix II. Same location and date as preceding.

(2): Road to Mesa Wind Area, east of Cottonwood Canyon, near Pacific Crest Trail crossing, Riverside Co., CA. 33° 56' 57.5"N 116° 40' 59.2"W, 02/02/2013. (1): Specimen lost, Mission Creek Canyon approx. 6.7 km. above Mission Creek Preserve lower parking lot, San Bernardino Co., CA. 34° 02' 50"N 116° 39' 32"W, 04/03/2011.

(1): foothills of Mt. San Jacinto, 5.7 km. SE of Cabazon, Riverside Co., CA. 33° 53' 48.7"N 116° 43' 48.3"W, 02/07/2017.

Group 4: *Eremarionta rowelli bakerensis* (2): GenBank KY986350, Vicinity of type location, base of limestone hill 2 km NW of Hwy 127 exit/115, Baker, San Bernardino Co., CA. 35° 16' 39"N 116° 05' 11"W, 02/26/2013.

Eremarionta "Travertine Pt." (2): GenBank KY986351, KY986352, base of Santa Rosa Mtns., 1.8 km W of Monterey Ave. exit, Hwy 86, Desert Shores, Imperial Co., CA. 33° 24' 08"N 116° 03' 52"W, 01/27/2013.

Group 5: *Eremarionta/Cahuillus* "Martinez Canyon" (2): GenBank KY986353, KY986354, Northern base of isolated rocky hill 1.1 km. W of the end of 72nd St., Thermal, Riverside Co., CA. 33° 31' 33"N 116° 11' 39"W, 11/21/2011.

Eremarionta/Cahuillus "Bear Creek Canyon" (2): GenBank KY986355, KY986356, 40 meters in from mouth of incised reach of Bear Creek Canyon, 2.5 air miles SW of Trailhead on Calle Tecate, La Quinta, Riverside Co., CA. 33° 37' 43.3"N 116° 19' 27.3"W, 11/12/2011.

Group 6: *Eremarionta* "Pahrump" (1): GenBank KY986357, Spring Mtn. foothills, 560 m N of Carpenter Canyon Rd., Nye Co., NV. 36° 10' 19"N 115° 50' 00"W, 04/02/2013.

Eremarionta rowelli acus (1): GenBank KY986358, 10 miles S of Needles, side canyon E side of Hwy. 95, San Bernardino Co., CA. 34° 40' 44"N 114° 37' 16"W, 12/14/2011.

Eremarionta "Kingston" (1): GenBank KY986359, 200 meters E of Smith Spring, Kingston Mtns., San Bernardino Co., CA. 35° 47' 15"N 115° 59" 44"W, 11/29/2012. (1): GenBank KY986360, edge of Omega Mine, 2 km. ESE of Excelsior Mine Rd., Kingston Mtns., San Bernardino Co., CA. 35° 47' 12"N 115° 58' 19"W, 12/13/2012.

Group 7: *Eremarionta millepalmarum* (2): GenBank KY986361, KY986362, under rocks and palm fronds, Biskra Palms Oasis, Indio Hills, Riverside Co., CA. 33° 47' 23"N 116° 14' 58"W, 03/01/2012.

Eremarionta morongoana (1): GenBank KY986363, East Wide Canyon, side canyon 400 m NE of end of Hilltop Rd., 2.3 km. N of Dillon Road, Riverside Co., CA. 33° 55' 56"N 116° 22' 34"W, 01/23/2012. (1): GenBank accession pending, base of slope, 280 m. W of Cottonwood Springs Rd., Joshua Tree National Park, Riverside Co., CA. 33° 43' 06.7"N 115° 48' 47.1"W, 02/11/2015.

(2): GenBank KY986364, KY986365, small rockslide south side of Box Canyon Rd., across road from Shavers Well, Mecca Hills, Riverside Co., CA. 33° 37' 06"N 115° 55' 02"W, 04/16/2012.

(2): GenBank KY986366, KY986367, Base of hill, east branch of Yellow Spots Canyon, 1.7 km. N (by air) of Dillon Rd., Riverside Co., CA. 33° 43' 26.5"N 116° 01' 49"W, 04/02/2012.

(3): Specimen #1: GenBank KY986368, steep NW-facing slope, Fargo Canyon Rd., 2.9 km NE from Aqueduct Rd., Riverside Co., CA. 33° 45' 47"N 116° 04' 60"W, 03/03/2012. Specimen #2: GenBank KY986369, juvenile hatched from egg laid by adult collected at preceding location and date. Specimen #3: same collection data as preceding. (1): GenBank KY986370, under rock beneath shrubs, small embayment on east side of Whitewater Canyon, 900 m SE of Whitewater Canyon Preserve entrance, Riverside Co., CA. 33° 58' 58"N 116° 38' 58"W, 11/16/2011. (1): GenBank KY986371, base of conglomerate outcrop, west side of Super Canyon, Painted Hills, Riverside Co., CA. 33° 56' 54"N 116° 37' 31"W, 01/23/2012. (2): GenBank KY986372, KY986373, southern bank of Mission Creek Wash, 250 m SSE of Mission Creek Preserve gate, Riverside Co., CA. 33° 59' 55"W 116° 36' 44"W, 12/28/2011. (1): GenBank KY986374, south side of mouth of Little Morongo Canyon, 1.7 km. (by air) NE of intersection of Mission Lakes Blvd. and Little Morongo Rd., Riverside Co., CA. 33° 59' 25.5"N 116° 31' 11"W, 01/09/2012.

Appendix II. Museum Voucher Specimens

1. *Sonorelix baileyi* — LACM 178951. GenBank KY986344, shell, SEM shell, preserved (EtOH) soft anatomy (minus reproductive system), and slide-mounted reproductive system (all but the SEM shell are the same individual). In talus, south side of Old Spanish Trail, Emigrant Pass, Nopah Mountains, Inyo Co., California, 35° 53' 10"N 116° 04' 13"W. Coll. D. Goodward, 19 December 2012. Slide: L. Gilbertson.
2. *Helminthoglypta (Coyote) greggi* — LACM 178952. GenBank KY986340, SEM shell and slide-mounted reproductive system. Southernmost outcrop of Soledad Mtn., 0.75 km. N. of Backus Rd., 0.41 km. E of 40th St., Kern Co., 34° 57' 27.4"N 118° 11' 55.8"W. Coll: D. Goodward, 3 September 2012. Slide: L. Gilbertson.
3. *Helminthoglypta (C.) micrometalleoides* — LACM 178953. GenBank KY986343, slide-mounted reproductive system. Topotype, "S side Iron Canyon Rd., 3 mi. up canyon from junction with Garlock-Goler

highway", El Paso Mtns., Kern Co., CA. 35° 26' 36"N 117° 47' 32"W. Coll: D. Goodward, 13 April 2013. Slide: L. Gilbertson.

4. *Chamaearionta aquaealbae* — LACM 178954. GenBank KY986348, SEM shell.
   Under *Chilopsis linearis* trees, access road adjacent to Cottonwood Wash, 1.3 km NNE of Haughen-Lehmann Way Exit, I-10, Riverside Co., 33° 56' 08"N 116° 41' 12"W. Coll: D. Goodward, 9 April 2013.